IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Karl-Heinz Bozung *et al.* Examiner: Helen Nguyen

Serial No.: 09/568,880 Group Art Unit: 1617

Filed: May 9, 2000 Docket: 1/1088

For: NEW MEDICAMENT COMPOSITIONS BASED ON ANTICHOLINERGICALLY-EFFECTIVE COMPOUNDS AND β-MIMETICS

Assistant Commissioner for Patents
Washington DC 20231

DECLARATION OF ALEXANDER WALLAND UNDER 37 C.F.R. § 1.132

Sir:

I, Alexander Walland, declare that:

1. I have studied Biology and Chemistry from 1960 to 1966 at the University of Vienna, Austria, and received a Ph.D. in Biology from the University of Vienna in 1966 and a Dr.rer.nat in Pharmacology at the University of Amsterdam, Netherlands, in 1979.

2. In 1982, I started teaching Pharmacology at the Medical Faculty of the Philipps University in Marburg, Germany, where I became in 1985 Privatdozent and in 1998 Professor in Pharmacology and Toxicology. Between January 1994 and December 1999, I was the Secretary General of the German Society for Experimental and Clinical Pharmacology and Toxicology.

3. From January 1962 to July 1966, I worked as a technician, and subsequently until July 1973, as a head of a pharmacological laboratory, in the Ernst Boehringer Institute for Drug Research in Vienna, Austria. From July 1973 to the present time, I have been employed in the Department of Pharmacology of Boehringer Ingelheim Pharma KG in Ingelheim, Germany, as a head of a laboratory group.

4. I am a coinventor of the above-identified patent application and I am familiar with the above-identified patent application.

5. I am familiar with the U.S.P.T.O. office action dated January 26, 2001, and the prior art references cited therein: Marecki *et al.* '539 (U.S. Patent No. 5,290,539), Jager *et al.* '930 (U.S. Patent No. 5,676,930), and Garvey *et al.* (International Publication No. WO 97/34871 assigned to Nitromed Inc.).

6. Under my responsibility and control, investigations with inhalative administration of the bronchospasmolytic test compounds tiotropium bromide and salmeterol hemisulfate in anaesthetized dogs were conducted according to the following protocol. In anaesthetized (pentobarbitone) dogs with artificial ventilation (suxamethonium) and instrumentation for measurement of pulmonary and cardiovascular function, test bronchospasms were precipitated by intravenous injection of acetylcholine at intervals of 30, 15, and 0 minutes before, and 1, 5, 10, 20, 30, 60, 120, and 180 minutes after, inhalative administration of the respective bronchospasmolytic test compounds. In 5 separate groups of 6 dogs each, the bronchospasmolytic test compounds were administered during inspiration into the orotracheal tube by means of a Respimat® nebulizer device as follows: (a) 3 tiotropium bromide, (b) 6 µg tiotropium bromide, (c) 6 µg salmeterol hemisulfate, (d) 12 µg salmeterol hemisulfate, and (e) a combination of 3 µg tiotropium bromide and 6 µg salmeterol hemisulfate.

7. The results of experimental tests according to the protocol of paragraph 6 herein were as follows. The bronchospasmolytic effect of salmeterol hemisulfate alone (groups (c) and (d)) was already maximal after 1 to 5 minutes and dissipated nearly completely within 180 minutes. In contrast, the bronchospasmolytic effect of tiotropium bromide alone (groups (a) and (b)) developed slowly, a maximum was achieved after 60 minutes only, but was almost completely maintained during the whole observation period. The combination of salmeterol and tiotropium bromide (group (e)) showed a fast onset of bronchospasmolytic effect and the effect was essentially maintained during the entire 180 minutes. The mean inhibition of bronchospasm calculated over 180 minutes after administration of the test compound was dose related for salmeterol hemisulfate alone (6 µg: 21.0 ± 3.24%; 12 µg: 31.5 ± 3.82%) as well as for tiotropium bromide alone (3 µg: 34.2 ± 1.80%; 6 µg: 57.0 ± 1.1%). The combination of 3 µg tiotropium bromide with 12 µg salmeterol hemisulfate induced a mean inhibition of 62.3 ± 3.61% and was

significantly more potent than the inhibition effect induced with 6 µg or 12 µg salmeterol hemisulfate or 3 µg tiotropium bromide administered alone. The bronchospasmolytic effect of the combination was also more pronounced than the calculated sum of the single components (48.0 ± 2.57%). Salmeterol hemisulfate increased heart rate by 11.0 beats/minute at 6 µg and 11.3 beats/minute at 12 µg (calculated over 60 minutes) while tiotropium bromide did not increase heart rate. The mean tachycardic effect of the combination of 6 µg salmeterol hemisulfate with 3 µg tiotropium bromide was smaller (3.6 beats/minutes) than that of 6 µg salmeterol hemisulfate administered alone and this difference was statistically significant.

8. To summarize the experimental results above, the combination 3 µg tiotropium bromide with 12 µg salmeterol hemisulfate induced a mean inhibition of bronchospasm of 62.3 ± 3.61% and was significantly more potent than the bronchospasmolytic effect achieved using 6 µg or 12 µg salmeterol hemisulfate or 3 µg tiotropium bromide alone. Furthermore, the bronchospasmolytic effect of the combination was greater than the calculated sum of the effects of each single component administered alone (48.0 ± 2.57%) and the bronchospasmolytic effect of the combination is therefore synergistic. Moreover, whereas salmeterol hemisulfate increased heart rate, the mean tachycardic effect of the combination of 6 µg salmeterol hemisulfate with 3 µg tiotropium bromide, calculated over 60 minutes, was smaller (3.6 beats/minutes) than that of 6 µg salmeterol hemisulfate administered alone and this result was statistically significant.

9. From the above experiments and results, I conclude that the combinations according to the invention of the above-identified patent application, as exemplified by salmeterol hemisulfate and tiotropium bromide, display an unexpected beneficial and synergistic effect which is greater than the calculated sum of the effects of each single component administered alone.

10. From the above experiments and results, I further conclude that the combinations according to the invention of the above-identified patent application cause less tachycardia or increased heart rate as a side effect than the administration of beta-mimetics alone.

11. Furthermore, I conclude that these surprising properties of the combinations according to the invention of the above-identified patent application were neither taught, suggested, nor deducible by the cited prior art. Moreover, I conclude that these findings would have been both surprising and unexpected to one of ordinary skill in the art at the time the invention was made.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: June 1st 2001 Signature: A. Walland

(Alexander Walland)